

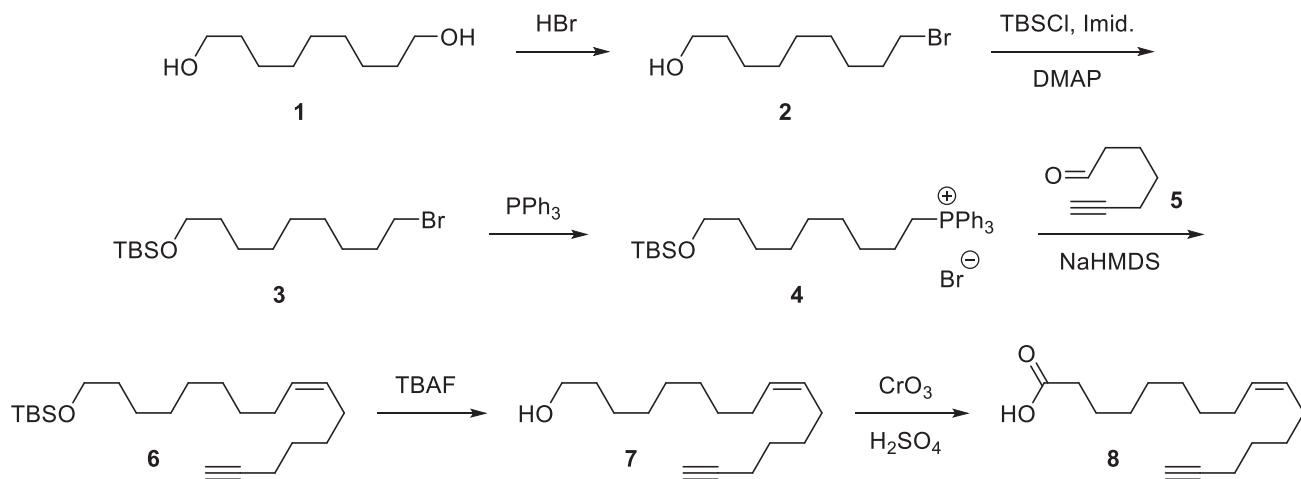
**Stereoselective fatty acylation is essential to the release of lipidated WNT proteins from the acyltransferase PORCN**

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**Supporting Experimental Procedures**

**Cis ω-alkynyl palmitoleic acid synthesis:**



**9-Bromononan-1-ol (2).** To a solution of 1,9-nanediol (**1**) (3.20 g, 20 mmol) in toluene (50 mL) was added 48% hydrogen bromide (28 mL, 160 mmol) at 0°C and then heated to reflux. After stirring overnight, the solution was cooled to 23°C, diluted with ethyl acetate (50 mL), and washed with saturated sodium chloride (50 mL). The aqueous layer was extracted by ethyl acetate (30 mL×2). The combined organic layers were dried over anhydrous sodium sulfate, concentrated and purified by silica gel column chromatography (25% ethyl acetate/hexanes) to provide **2** as a yellow solid (3.40 g, 76% yield). 1H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.64 (t, J = 6.4 Hz, 2H), 3.40 (t, J = 6.8 Hz, 2H), 1.86-1.82 (m, 2H), 1.57-1.54 (m, 2H), 1.44-1.30 (m, 10H).

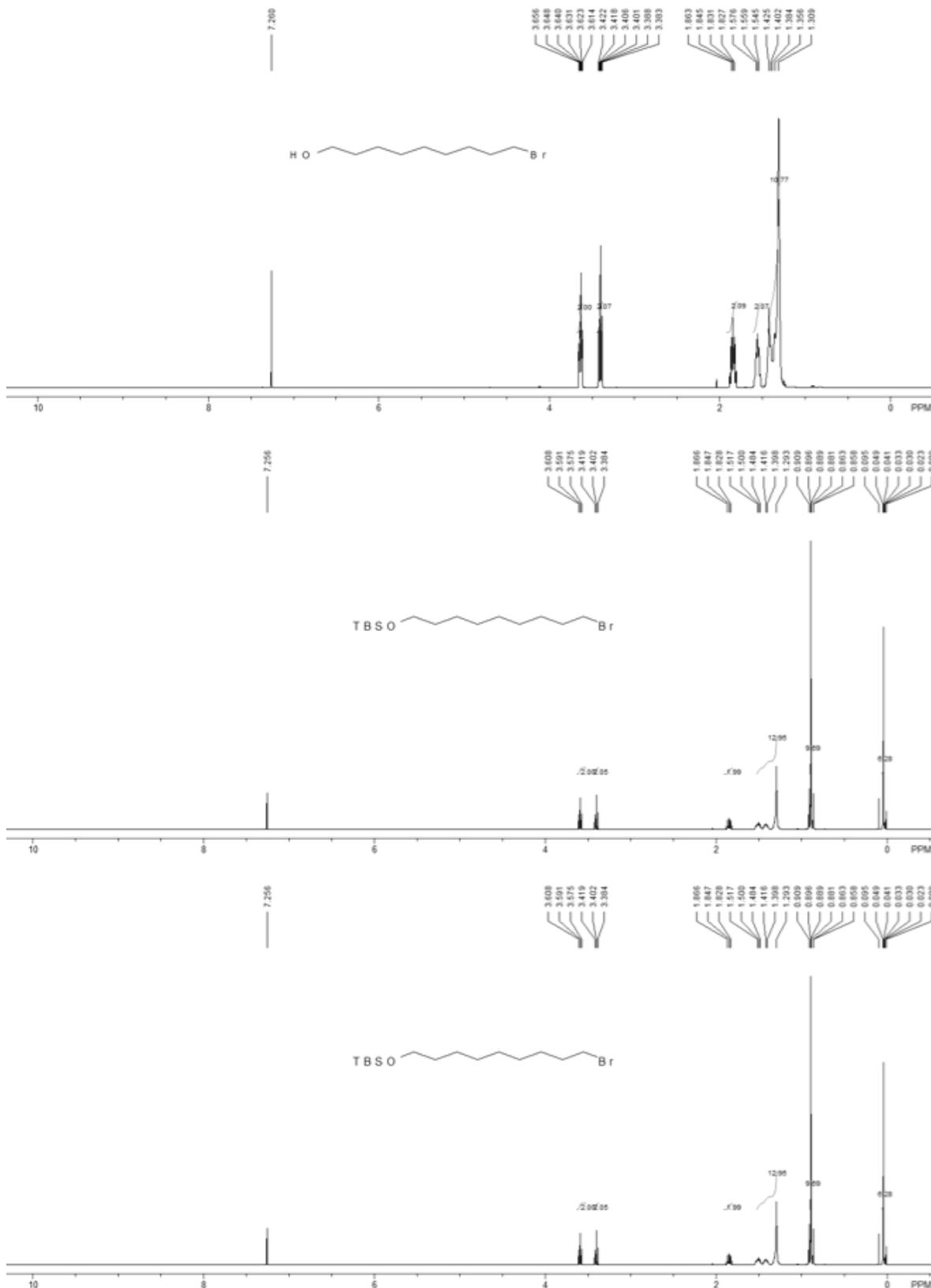
**9-(tert-Butyldimethylsiloxy)nonanyl bromide (3).** To a solution of **2** (3.40 g, 15.2 mmol) in dichloromethane (60 mL) were added imidazole (2.68 g, 39.5 mmol), 4-dimethylaminopyridine (185 mg, 1.5 mmol) and tert-butyldimethylsilyl chloride (2.98 g, 19.8 mmol) at 23°C. After stirring overnight, the solution was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography (25% ethyl acetate/hexanes) to provide **3** as a colorless oil (5.00 g, 98% yield). 1H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.59 (t, J = 6.4 Hz, 2H), 3.40 (t, J = 6.8 Hz, 2H), 1.86-1.82 (m, 2H), 1.54-1.29 (m, 12H), 0.89 (s, 9H), 0.04 (s, 6H).

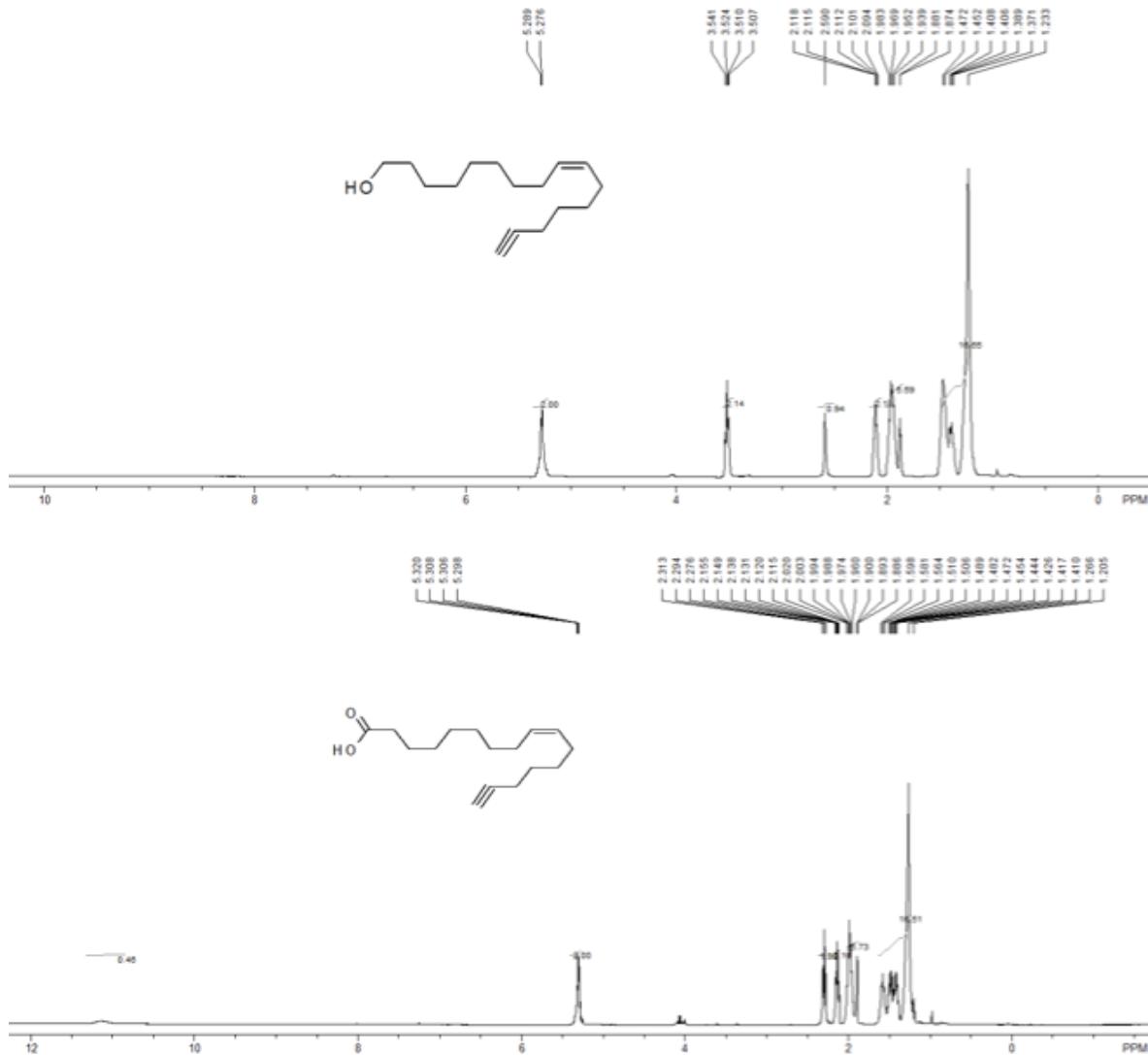
**9-(tert-Butyldimethylsiloxy)nonanytriphenylphosphonium bromide (4).** To a solution of **3** (3.0 g, 8.89 mmol) in acetonitrile (40 mL) was added triphenylphosphine (2.56 g, 9.78 mmol) and then heated to reflux. After stirring for two days, the solution was cooled to 23°C and concentrated to give crude **4**, which was used directly for the next step without purification.

**(Z)-tert-Butyl(hexadec-9-en-15-ynyoxy)dimethylsilane (6).** To a solution of **4** (1.3 g, 2.16 mmol) in tetrahydrofuran (15 mL) was added sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 2.0 mL) at 0°C followed by aldehyde **5** (183 mg, 1.67 mmol) in tetrahydrofuran (5 mL) dropwise at -78°C. After stirring at 23°C overnight, the reaction was quenched with saturated ammonium chloride solution and extracted with ethyl acetate (10 mL×3). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated to give crude **6**, which was used directly for the next step without purification.

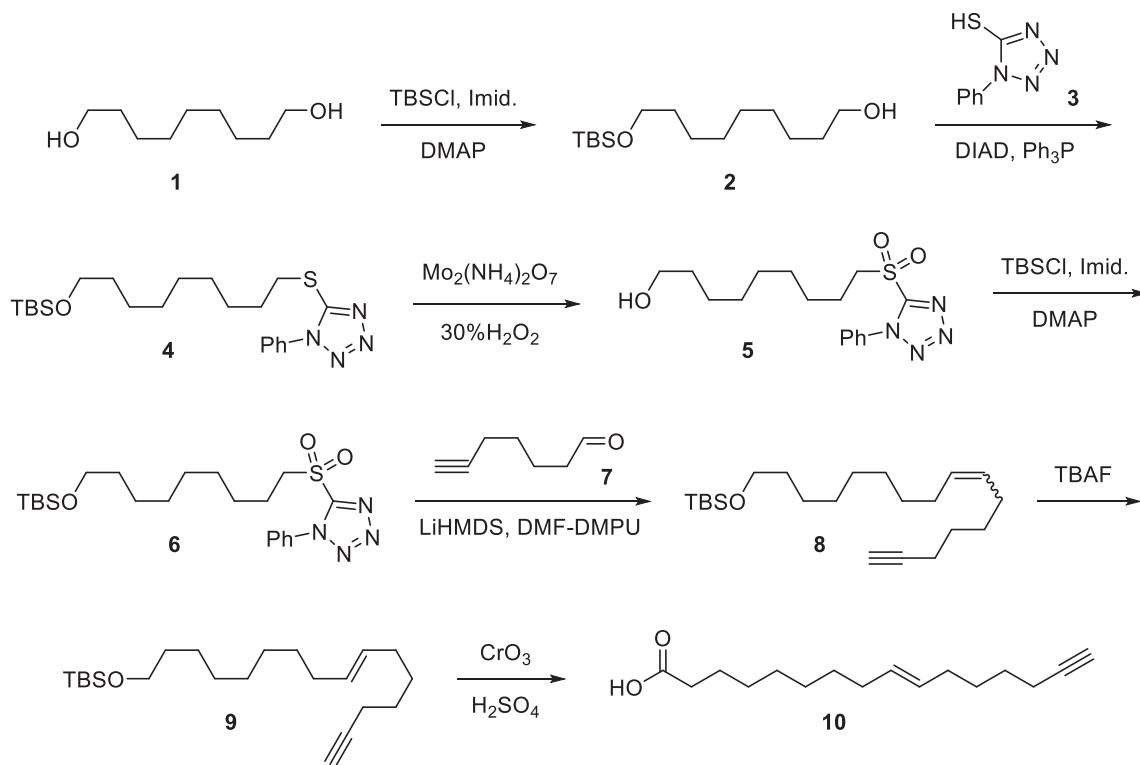
**(Z)-Hexadec-9-en-15-yn-1-ol (7).** To a solution of crude **6** in tetrahydrofuran (10 mL) was added tetra-n-butylammonium fluoride (1.0 M in tetrahydrofuran, 4.0 mL) at 23°C. After stirring for 4 hrs, the reaction was quenched with water and extracted with ethyl acetate (10 mL×3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography (15% ethyl acetate/hexanes) to provide **7** as a colorless oil (200 mg, 50% yield by two steps). 1H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.29-5.26 (m, 2H), 3.52 (t, J = 6.8 Hz, 2H), 2.59 (s, 1H), 2.11-1.87 (m, 7H), 1.47-1.23 (m, 16 H).

**(Z)-Hexadec-9-en-15-ynoic acid (8).** To a solution of 7 (200 mg, 0.846 mmol) in acetone (3 mL) was added chromium(VI) oxide (210 mg, 2.1 mmol) in 20% aqueous sulfuric acid (0.7 mL) dropwise at 0°C. After stirring for 30 min, the reaction mixture was diluted with water (10 mL) and extracted by ethyl acetate (10 mL×3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated and purified by silica gel column chromatography (25% ethyl acetate/hexanes) to provide 8 as a colorless oil (130 mg, 62% yield).  $^1\text{H}$  NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  11.1 (brs, 1 H), 5.32–5.28 (m, 2 H), 2.29 (t,  $J$  = 7.2 Hz, 2H), 2.13 (dt,  $J$  = 7.2, 2.4 Hz, 2H), 2.02–1.88 (m, 5H), 1.15–1.26 (m, 14 H).





**Trans  $\omega$ -alkynyl palmitoleic acid synthesis:**



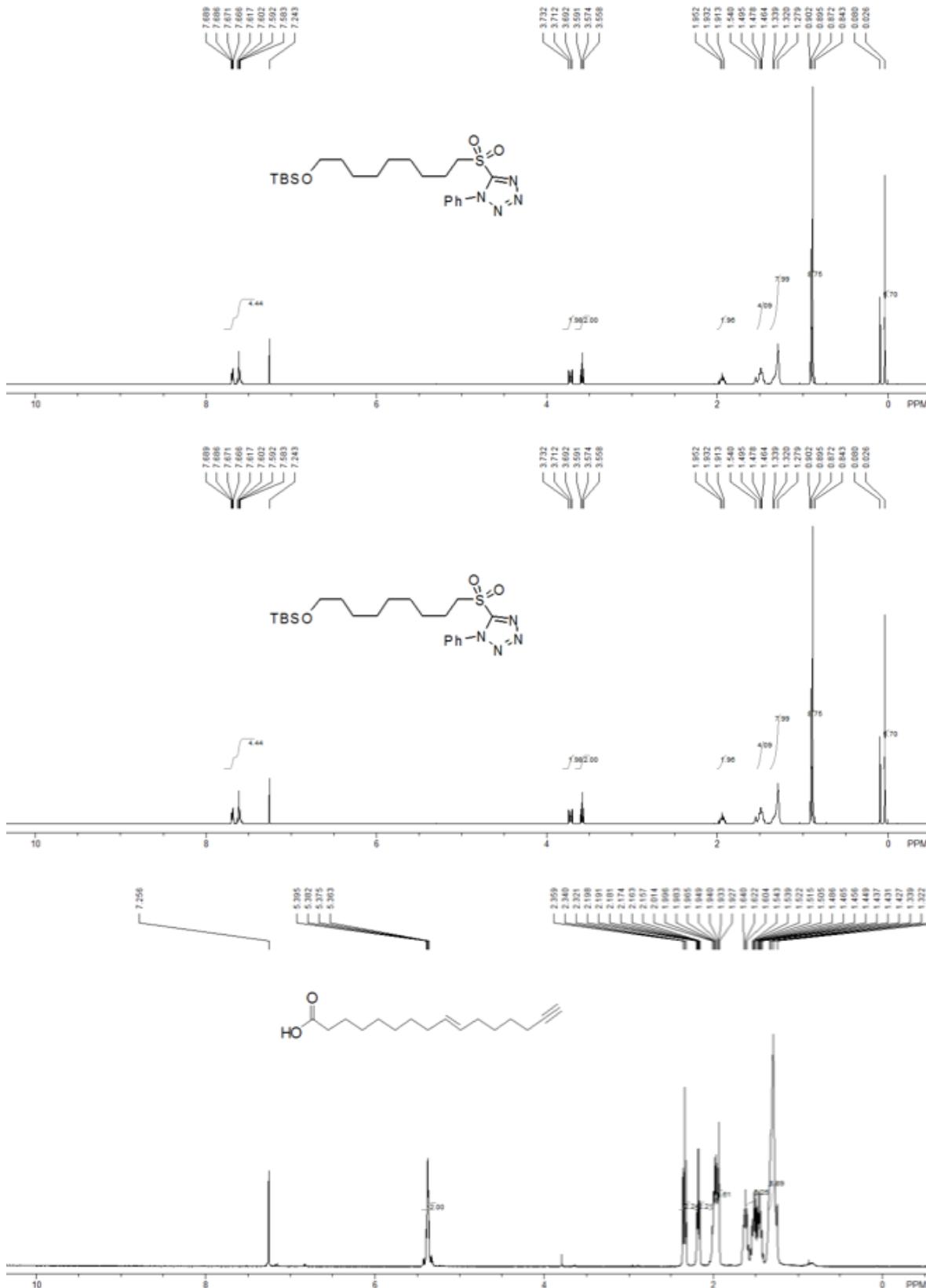
**9-(tert-Butyldimethylsilyloxy)nonan-1-ol (2).** To a solution of nonane-1,9-diol 1 (3.2 g, 20 mmol) in tetrahydrofuran (200 mL) was added imidazole (2.72 g, 40 mmol) and tert-butyldimethylsilyl chloride (3.01 g, 20 mmol) at 23°C. After stirring overnight, the solvent was removed and the residue was diluted with ethyl acetate (50 mL), washed with saturated sodium chloride. The organic layer was dried over anhydrous sodium sulfate, concentrated and purified by silica gel column chromatography (15% ethyl acetate/hexanes) to provide **2** as an oil (2.9 g, 53% yield).

**9-(1-Phenyl-1H-tetrazol-5-ylsulfonyl)nonan-1-ol (5).** To a solution of **2** (2.0 g, 7.28 mmol) in tetrahydrofuran (80 mL) was added 1-phenyl-1H-tetrazole-5-thiol (1.94 g, 10.93 mmol), triphenylphosphine (2.86 g, 10.93 mmol) and diisopropyl azodicarboxylate (2.14 mL, 10.93 mmol) at 23°C. After stirring for 2 h, the reaction mixture was diluted with ethanol (60 mL) and cooled to 0°C. To this solution was added a bright yellow solution of a mixture of 30% aqueous hydrogen peroxide (12 mL, 109.3 mmol) and ammonium molybdate (1.61 g, 1.09 mmol) dropwise. After stirring at 23°C overnight, the reaction was quenched with water and extracted with dichloromethane (80 mL × 3). The combined organic layers were washed with saturated sodium bisulfate, brine, dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was used directly in the next step without further purification.

**5-(9-(tert-Butyldimethylsilyloxy)nonylsulfonyl)-1-phenyl-1H-tetrazole (6).** To a solution of **5** in dichloromethane (80 mL) was added imidazole (1.48 g, 21.84 mmol), tert-butyldimethylsilyl chloride (1.64 g, 10.9 mmol) and 4-dimethylaminopyridine (89 mg, 0.72 mmol) at 23°C. After stirring overnight, the reaction was quenched with saturated sodium chloride and extracted with dichloromethane (50 mL × 3). The combined organic layers were dried over anhydrous sodium sulfate, concentrated and purified by silica gel column chromatography (20% ethyl acetate/hexanes) to provide **6** as an oil (2.3 g, 67% yield over three steps). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.69–7.58 (m, 5H), 3.71 (t, J = 8.0 Hz, 2H), 3.57 (t, J = 6.8 Hz, 2H), 1.95–1.91 (m, 2H), 1.49–1.46 (m, 4H), 1.34–1.27 (m, 8H), 0.87 (s, 9H), 0.02 (s, 6H).

**tert-Butyl(hexamadec-9-en-15-ynyl)dimethylsilane (8).** To a solution of **6** (953 mg, 2.04 mmol) in N,N-dimethylformamide (7.5 mL) and N,N'-dimethylpropylene urea (2.5 mL) was added lithium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 2.0 mL) slowly at -78°C. After stirring for 30 min, aldehyde 7 (150 mg, 1.36 mmol) in N,N-dimethylformamide (3 mL) and N,N'-dimethylpropylene urea (1 mL) was added dropwise. After stirring at 23°C overnight, the reaction was quenched with saturated ammonium chloride solution and extracted with ethyl acetate (10 mL × 3). The organic layers were washed with brine (10 mL × 2), dried over anhydrous sodium sulfate, filtered and concentrated. The crude product (Z/E = 1:1) was used directly for the next step without purification. (E)-Hexadec-9-en-15-yn-1-ol (**9**). To a solution of crude **8** in tetrahydrofuran (5 mL) was added tetra-n-butylammonium fluoride (1.0 M in tetrahydrofuran, 1.7 mL) at 23°C. After stirring for 4 h, the reaction was quenched with water and extracted with ethyl acetate (10 mL × 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated and purified by prepared HPLC to provide **9** as a colorless oil (64 mg, 20% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.39–5.36 (m, 2H), 3.66 (t, J = 6.8 Hz, 2H), 2.19–1.92 (m, 7H), 1.47–1.23 (m, 17 H).

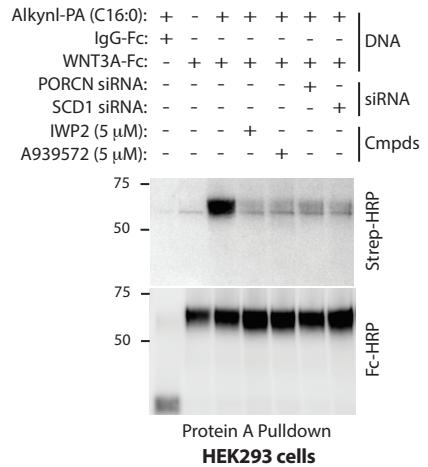
**(E)-Hexadec-9-en-15-yneic acid (10).** To a solution of **9** (30 mg, 0.127 mmol) in acetone (1 mL) was added chromium(VI) oxide (32 mg, 0.315 mmol) in 20% aqueous sulfuric acid (0.15 mL) dropwise at 0°C. After stirring at 0°C for 30 min, the reaction mixture was diluted with water (3 mL) and extracted by ethyl acetate (5 mL × 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated, and purified by silica gel column chromatography(25% ethyl acetate/hexanes) to provide **10** as a colorless oil (13 mg, 43% yield). 1H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.39-5.36 (m, 2 H), 2.34 (t, J = 7.6 Hz, 2H), 2.17 (dt, J = 6.8, 2.8 Hz, 2H), 2.01-1.92 (m, 5H), 1.64-1.24 (m, 14 H).



## Supplemental Figures:



**Supplemental Figure 1. PORCN sequence alignment across metazoan phyla.** Amino acid sequence alignment of PORCN proteins from human (*H. sapiens*), mouse (*M. musculus*), frog (*X. laevis*), zebrafish (*D. rerio*), roundworm (*C. elegans*), fruitfly (*D. melanogaster*), flatworm (*S. mansoni*), and chicken (*G. gallus*). Predicted transmembrane regions are shown as blue cylinders. A highly conserved histidine residue is highlighted in yellow.



**Supplemental Figure 2. SCD is essential for WNT acylation.** WNT3A-Fc labeling with a saturated alkynyl palmitic acid (C16:0) probe was abolished in the presence of a PORCN inhibitor (IWP2, 5  $\mu$ M) or SCD inhibitor (A939572, 5  $\mu$ M). RNAi-mediated gene knockdown of PORCN and SCD also prevented WNT3A-Fc labeling.